

Research article

CHEK2 I100delC is prevalent in Swedish early onset familial breast cancerSara Margolin¹, Hans Eiberg², Annika Lindblom³ and Marie Luise Bisgaard^{*4}

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Abstract

Background: A truncating variant, I100delC, in check point-kinase CHEK2, has been identified as a risk factor for familial and sporadic breast cancer. The prevalence in healthy non-breast cancer cases is low and varies between populations.

Methods: We analyzed the prevalence of CHEK2 I100delC in 763 breast cancer patients with a defined family history and 760 controls from the Stockholm region. The breast cancer patients originated from; a population-based cohort (n = 452) and from a familial cancer clinic (n = 311), the detailed family history was known in both groups.

Results: The variant was found in 2.9% of the familial cases from the population-based cohort and in 1.9% from the familial cancer clinic. In total 2.2% of the patients with a family history of breast cancer carried the variant compared to 0.7% of the controls (p = 0.03). There was no increased prevalence in sporadic patients (0.3%). The variant was most frequent in young familial patients (5.1% of cases ≤45 years, p = 0.003). The mean age at diagnosis of variant carriers was 12 years lower than in non-carriers (p = 0.001).

Conclusion: In conclusion, CHEK2 I100delC exists in the Swedish population. The prevalence is increased in familial breast cancer and the variant seems to influence age at onset.

Background

Apart from gender, family history is the most important risk factor for breast cancer. Mutations in the known high-risk genes *BRCA1*, *BRCA2*, *p53*, *ATM* and *PTEN* account for less than 25% of the familial risk for breast cancer while the remainder are still genetically unexplained despite large efforts in research [1]. A polygenic model with variations in several loci, each contributing a modest independent risk has been shown to best explain the residual non *BRCA1/2* aggregation of breast cancer, and

the effect of low penetrant genes may also at least partly explain sporadic breast cancer [2,3]. However, there are few conclusive results on variants in candidate low-penetrant genes even though a large number of case-control studies, most relatively small in sample size, have been performed [4]. Association studies on variants conferring modest risks require large sample sizes; the study size is however also influenced by the variant frequency. In general unselected breast cancer cases are used for association studies, but the study size can be reduced by selecting

cases enriched for genetic susceptibility such as patients with family history or bilateral cases [5]. A rare truncating variant in *CHEK2* (1100delC), a G2 checkpoint-kinase, that is involved in cell cycle control and DNA-repair, was identified as a risk factor for breast cancer in two independent studies using this approach [6-8]. The variant frequency was assessed in high-risk familial non *BRCA1/2* cases and healthy controls, and both studies were highly significant despite the fact that the variant is rare (1% or less in normal population) and confers a relative risk of around 2 [7,8]. More than 600 unselected cases were tested in each study but no significant difference in allele frequency between unselected cases and controls was detected [7,8]. The variant was suggested to be a low-penetrance gene as described in the polygenic model but the possibility of a role as a modifier of as – yet unknown high-risk gene/s has also been proposed [7-9]. A subsequent collaboration study of more than 10 000 cases and controls has demonstrated a doubled risk also in unselected cases carrying the variant [10]. In the present study we analyse the importance of the variant among breast cancer patients with a known familial disposition compared to sporadic cases and controls. Furthermore, we evaluate the significance of the variant as a modifier gene versus a low-penetrance gene.

Methods

Material

Familial Risk Cohort

311 independent familial cases collected at the Department of Clinical Genetics at Karolinska Hospital, Stockholm were used for the study. These patients had either been referred due to a breast cancer diagnosis and a family history of breast cancer or had been collected as part of previous research on familial breast cancer [11]. All cases had proceeded through genetic counseling and almost all (>90%) had been screened negative for *BRCA1/2* mutations (those who met the current criteria for screening and the majority of the others as part of previous research to define criteria for screening). *BRCA1* and *BRCA2* mutations are rare in the Stockholm region and in previous studies only 1% mutations in each gene were detected in familial breast cancer if not using age criteria [12,13].

For the Familial Risk cohort only age at diagnosis was available (missing in 16 patients).

Population-based cohort

Patients with a surgically treated primary invasive breast cancer admitted to the Department of Oncology at Huddinge Hospital and Söder Hospital (covering the population of southern Stockholm of 850 000 people) from October 1998 to May 2000 were asked participate in a study on genetic risk factors from breast cancer [14]. Family history, age at diagnosis, hormone receptor status and

histology of the tumor were obtained from all cases and the median follow-up was 5 years. This cohort consists of 489 patients in total and 456 were used in this study due to logistic reasons. The samples had previously been screened for mutations in exon 11 of *BRCA1* where more than 70% of the mutations, including four founder mutations, identified in the Stockholm region are found [14]. Four cases with known *BRCA1* or *BRCA2* mutations were excluded from the study.

Cases with at least one 1st or 2nd-degree relatives with breast cancer in addition to the proband, regardless of age, were classified as familial breast cancer. In the Familial risk cohort 168 cases had one 1st or 2nd degree relative, while the remainder had more than one 1st or 2nd degree relative. In the population-based cohort 104 cases had one relative and 35 more than one relative with the disease.

The mean age at diagnosis in the Familial risk cohort was 54 years (24–92 years). In the Population-based cohort the mean age was 60 years (27–88 years) and there was no statistically significant difference between familial and sporadic cases (59 and 61 years respectively).

As controls we used DNA from 760 geographically matched blood-donors of mixed gender collected as a control material for association studies at Karolinska University Hospital, Stockholm, Sweden.

The Ethical Committee at the Karolinska Institute approved the study.

Methods

Genotyping of the most common mutation in the *CHEK2* gene (1100delC) was performed on controls and patients by PCR with a primer set that is specific for the mutation (M-*CHEK2*del100C-F: 5'-gca aag aca tga atc tgt aaa gtc-3' M-*CHEK2*del100C-R: 5'-aaa tct tgg agt gcc caa aat aat-3' and a primer set specific for the wild type allele: (W-*CHEK2*1100delC-F: 5'-gca aag aca tga atc tgt aaa gtc-3' and 3' W-*CHEK2*1100delC-R: 5'-aaa tct tgg agt gcc caa aat cag-3' resulting in 184 base pair products. DNA amplifications were carried out in a 20 µl volume containing 1 × Ampli-con III standard buffer (Ampliqon ApS, Copenhagen), 125 µM of each dNTP, 4 pmol of each primer, 0.5 units of Ampli-con III Taq Polymerase (Ampliqon ApS, Copenhagen), and 25 – 50 ng of template DNA. The PCR conditions were: initial denaturation at 95°C for 5 min. hereafter 40 cycles at 95°C for 20 sec, 59°C for 20 sec and 72°C for 20 sec followed by 5 min. of extension at 72°C.

The two reactions were run separately each in multiplex with a control PCR (Control-F: 5'-gtc aaa gcc acc agt tac agt-3' and Control-R: 5'-ttc ccc acc act tta ctg ac-3') result-

Table 1: Prevalence of CHEK2 1100delC in sporadic and familial breast cancer and controls

| | CHEK2 1100delC+/total tested | p-value ¹ |
|--------------------------------|------------------------------|----------------------|
| Familial Risk Cohort | 6/311 (1.9%) | 0.09 |
| Population-based Cohort | 5/452 (1.1%) | 0.51 |
| Sporadic breast cancer | 1/313 (0.3%) | 0.68 |
| Familial Breast Cancer | 4/139 (2.9%) | 0.04 |
| All Familial patients | 10/450 (2.2%) | 0.03 |
| Controls | 5/760 (0.7%) | |

¹P-values were calculated with Fisher's test for association

ing in a product of 309 base pairs on chromosome 4. The products were separated on 2.1% SeaKem LE Agarose (Cambrex, Bio Science Rockland, Inc., Rockland, ME, USA) gels. In order to examine for homozygosity for the variant, PCR for wild type allele and control were performed in cases with the CHEK2 1100delC variant.

Statistical analysis

Fisher's exact test was used to compare categorical data. Continuous data were compared using the two-sample t-test.

Results

The prevalence of CHEK2 1100delC was 1.9% in the familial risk Cohort and 1.1% in the population-based cohort compared to 0.7% in the controls ($p = 0.09$ and 0.51 respectively). 2.2% of all the familial cases carried the variant ($p = 0.03$), corresponding to an odds ratio of 3.4 (95% CI 1.2–10.1). (Table 1). Only one of the sporadic cases carried the variant (0.3%). There was an increasing frequency of the variant with decreasing age at diagnosis in the familial patients (Table 2).

The mean age at diagnosis of variant carriers was 12 (familial risk cohort) and 10 (population-based cohort) years lower in carriers than in non-carriers; 42 (24–55) vs. 54 years in familial risk cohort ($p = 0.01$) and 50 years (38–65) versus 60 years in the population-based cohort ($p = 0.06$). If the groups were combined the difference in mean age at onset was 12 years (46 vs. 58 years, $p = 0.001$).

In three families of carriers from the Familial Risk cohort, DNA was available for more family members. In two of these families, the mutation carrier status did not segregate with disease (Figure 1).

The five heterozygotes in the Population-based cohort all had unilateral ductal breast cancer without recurrence or secondary malignancies with 6 years follow-up.

Only one CHEK2 1100delC carrier did not have a family history of breast cancer (Table 3).

Discussion

We have evaluated the prevalence of the CHEK2 1100delC variant in two cohorts of breast cancer patients from the Stockholm region, one familial and one population-based, both with a well-defined family history and in controls. In our study CHEK2 1100delC was associated with familial breast cancer (2.2% vs. 0.7% in controls, $p = 0.03$) and confirm previous results of the variant as a risk factor for breast cancer.

In the original studies from Finland and the Netherlands the prevalence in familial breast cancer was 3.1–5.5 % [7,8]. The highest prevalence of CHEK2 1100delC has been reported in familial non-BRCA1/2 families also harboring colon cancer cases (18%) and in some highly selected high-risk breast cancer families (9–11%) [9,15,16]. The prevalence varies according to ethnicity and in studies mainly from Central and southern Europe, the variant, even in high-risk families, is very rare (<1%) [17–20].

Sporadic breast cancer cases have been reported to have a higher prevalence of the variant than healthy controls as demonstrated in the large pooled study by the Breast Cancer Consortium (1.9% vs. 0.75%) [10]. In our study, we found no accumulation of CHEK2 1100delC among sporadic cases, the cohort however too small to draw any firm conclusions. The difference in prevalence in our two cohorts regarding familial breast cancer (1.9% vs. 2.9%) is also based on very few cases and we refrain from analyzing this difference.

The prevalence of CHEK2 1100delC in our controls was 0.7%, which is consistent with previous studies in the Swedish population (0.4–1%) and the large pooled control material of more than 9000 controls, mainly of Western European origin (0.7%) [10,21–23].

In our material the CHEK2 1100delC carriers were markedly younger at diagnosis compared to non-carriers, even though the difference was of borderline significance in the two groups of breast cancer cases and the material small. Several previous studies support this finding including the

Table 2: Prevalence of CHEK2 1100delC in familial breast cancer according to age at diagnosis compared to controls (Familial Risk Cohort and Population-based cohort, age at diagnosis missing in 16 patients)

| Age at diagnosis, years | CHEK2 1100delC+/total tested | p-value ¹ |
|-------------------------|------------------------------|----------------------|
| <45 | 5/98 (5.1%) | 0.003 |
| 46–55 | 4/148 (2.7%) | 0.04 |
| >55 | 1/188 (0.5%) | 1.0 |

¹P-values were calculated with Fisher's test for association

large pooled analysis where mutation prevalence decreased with increasing age at diagnosis [9,10,15,24–26]. Other studies have found a modest, non-significant, difference regarding age at onset and there are also negative studies including the original Dutch study [7,8,16,27,28]. In the Dutch study, however, only familial cases were included and the mean age in both carriers and non-carriers was 45 years, which was much lower than in breast cancer patients in general, resulting in decreased power for identification of differences in age at onset. In a recent Swedish study on postmenopausal breast cancer there was 0.7% 1100delC carriers in cases compared to 0.4% in controls, which is consistent with our data on older patients even with a family history (Table 2) since the majority of our mutation carriers were diagnosed premenopausally [23].

CHEK2 was originally suggested to be a high-risk gene for the Li Fraumeni syndrome [29]. There is limited or no co-segregation of CHEK2 1100delC and disease in families both in our and other previous studies which contradicts this role of CHEK2 [7–9,16,30,31]. The role for the CHEK2 1100delC variant might then either be a low risk

variant on its own, or constitute a modifier of risk in syndromes with as yet unknown high-risk gene(s).

Our results, generated in a material with a well-defined family history including the paternal side, support the role of a modifier, as there was no accumulation of the variant in true sporadic cases, and the variant seemed to influence the age at diagnosis in carriers. Our material is however relatively small, and this conclusion would need to be verified in a larger material.

Conclusion

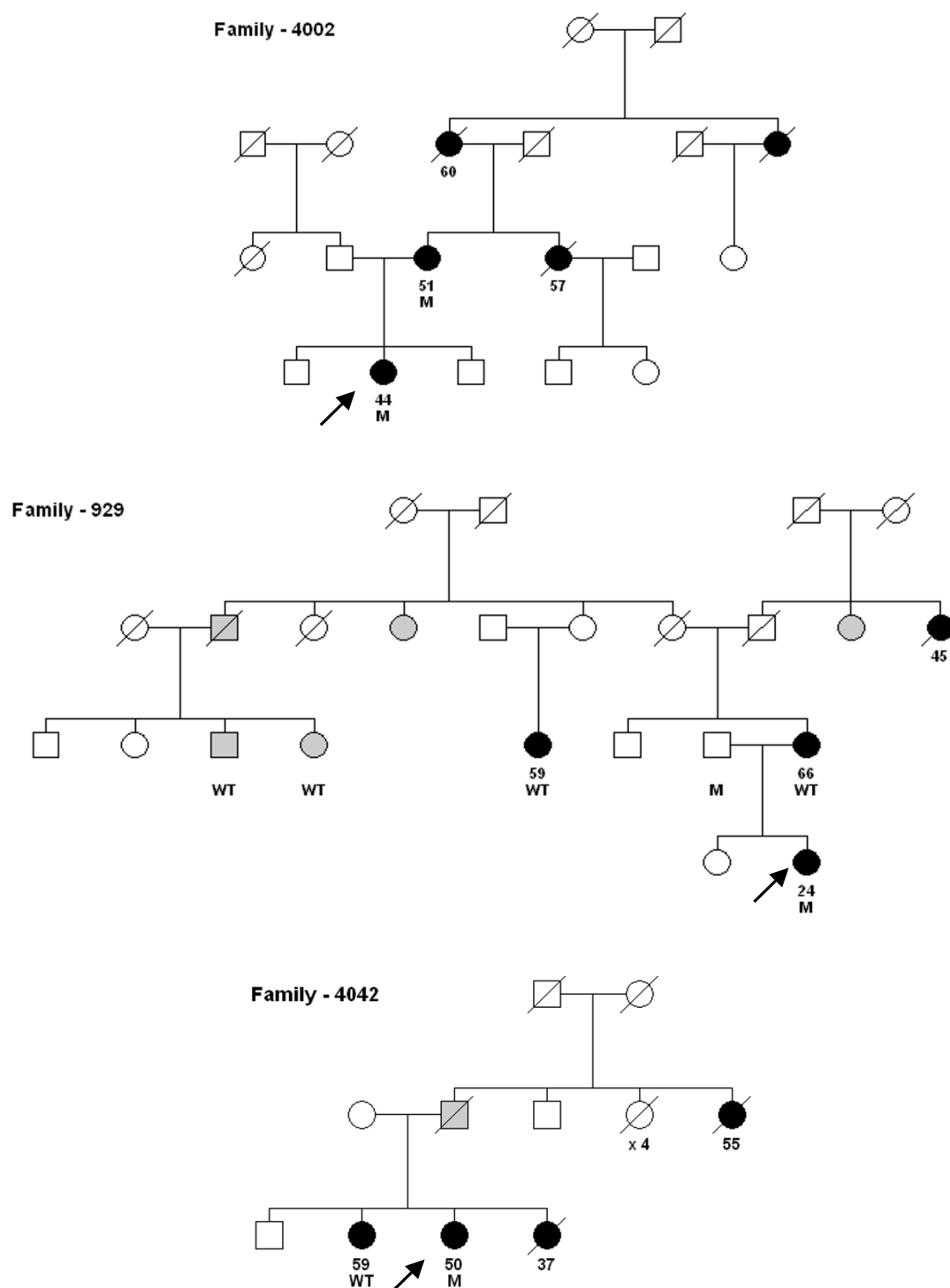
In conclusion the CHEK2 1100delC variant was significantly more frequent in familial cases assumed to modify an underlying hereditary fault. Since the effect of the variant is modest and the variant rare, there is no need for CHEK2 1100delC screening at present but the variant might prove interesting in combination with other genetic/non-genetic factors in the future.

Competing interests

The author(s) declare that they have no competing interests.

Table 3: Family history of the CHEK2 1100delC carriers (dr = degree relative/s)

| Family number and age at diagnosis | Breast Cancer | Other malignancies |
|------------------------------------|---|--|
| 102, 65 | Five 1 st dr including one male, age 40–67 | One 1 st dr with cancer uteri |
| 195, 54 | | One 1 st dr with brain tumour |
| 510, 41 | One 2 nd dr | |
| 764, 52 | One 1 st dr, age 28 | |
| 875, 38 | One 1 st dr, age 36 | One 1 st dr with non-Hodgkin lymphoma |
| 929, 24 | One 1 st dr, age 66 | One 2 nd dr with colon cancer |
| | One 3 rd dr, age 45 | One 3 rd dr with pancreatic cancer, Two 3 rd dr with cancer of the abdomen |
| | One 4 th dr, age 59 | |
| 1902, 55 | One 2 nd dr, age 75 | One 1 st dr with gyn. malignancy |
| | | One 2 nd dr with cancer of the abdomen |
| | | One 2 nd dr with sarcoma |
| 4002, 44 | One 1 st dr, age 51 | |
| | Two 2 nd dr, age 57, 60 | |
| | One 3 rd dr | |
| 4042, 50 | Two 1 st dr, age 37, 53 | One 2 nd dr with pancreatic cancer |
| | One 2 nd dr, age 55 | |
| 5031, 46 | One 1 st dr, age 44 | |
| 5611, 30 | Two 2 nd dr, age 58, unknown | One 2 nd dr with cancer uteri |
| | One 3 rd dr, age 51 | |

**Figure 1**

Pedigrees of families, arrow = index case. Black symbols are breast cancer cases; gray symbols are cases with any other cancer and the number below the symbols are age at diagnosis. WT = CHEK2 1100delC mutation, M = CHEK2 1100delC carrier.

Authors' contributions

SM collected the population-based material, participated in the design of the study, performed the statistical analysis and wrote the manuscript.

HE participated in development of the method and in the molecular genetic analysis.

AL conceived the idea of the study, collected the familial risk cohort material and participated in the analysis of the study.

MLB participated in the design of the study, performed the molecular genetic analysis, and participated in the analysis of the results and in drafting the manuscript.

All authors read and approved the final manuscript

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